

**An Investigation of a Sono-Chemical  
Approach in Sterilization Problems**

**Fourth Semi-Annual Progress Report**

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### 1. Purpose of Investigation.

The possible use of air-borne sound in sterilization problems confronting NASA is continuing. Studies on the effect of sonic and ultrasonic frequencies, propagated in air, on spores of B. subtilis var. niger have already been reported. In addition, the combined effects of sound and ethylene oxide have been noted in past reports.

The present report contains data on the antimicrobial effects of ultrasonic waves at 60 C and at 30 C. The use of propylene oxide in combination with ultrasonic waves is also included.

### 2. Alterations in Procedures Employed.

The test apparatus itself is identical to that described in the Third Semi-Annual Report (1 July 1965 - 31 December 1965). The change instituted presently involves the use of propylene oxide in place of ethylene oxide. This change was made because of the difficulties encountered with the use of ethylene oxide in our test apparatus. The higher boiling point of propylene oxide as compared to that of ethylene oxide eliminates the problem of volatilization experienced with the latter compound.

### 3. Effect of Ultrasonic Irradiations on the Spores of B. subtilis var. niger at 60 C and 30 C.

In the Third Semi-Annual Progress Report (1 July 1965 - 31 December 1965) data were presented for the irradiation of spores at a distance of 1 inch from the transducer at 60 C. Since the last report, additional experiments have been run and the results are included in the final figures. As a result, slight differences in per cent kill will be noted. Table 1, therefore, contains a repetition of data from the previous report ( 1 inch irradiation) and it also

presents new data compiled from experiments on the irradiation of spores at other distances at 60 C.

Table 1. Average counts ( $\times 10^6$ ) and % reduction in viable counts (in parentheses) of *B. subtilis* var. *niger* spores exposed to ultrasonic irradiation ( $34.8 \text{ kw/sec}$ ) at 60 C.

Hours	Distance from Transducer, inches			
	9.5	5.0	3.5	1.0
1	1.50 (0%)	1.42 (0%)	1.45 (0%)	1.30 (8%)
2	1.36 (4%)	1.32 (6%)	1.40 (1%)	1.24* (12%)
4	1.36 (4%)	1.27 (10%)	1.27 (10%)	0.96* (32%)
8	1.26 (11%)	1.14 (19%)	1.19 (16%)	0.91* (36%)

\* Denotes statistically significant value at 99% level of certainty.

In order to more fully evaluate the role of temperature on the antimicrobial effects of ultrasound on *B. subtilis* var. *niger*, the experimental design was expanded to include irradiations at 30 C. Table 2 summarizes the results obtained:

Table 2. Average counts ( $\times 10^6$ ) and % reduction in viable counts (in parentheses) of *B. subtilis* var. *niger* spores exposed to ultrasonic irradiation ( $34.8 \text{ kw/sec}$ ) at 30 C.

Hours	Distance from Transducer, inches			
	9.5	5.0	3.5	1.0
1	1.35 (5%)	1.47 (0%)	1.29 (9%)	1.26 (11%)
2	1.37 (3%)	1.25 (11%)	1.40 (1%)	0.94* (33%)
4	1.20 (15%)	1.16 (18%)	0.75* (47%)	0.92* (35%)
8	0.72* (49%)	0.76* (46%)	0.83* (41%)	0.67* (52%)

\* Denotes statistically significant value at 99% level of certainty.

Comparing the results obtained at 60 and 30 C presented here with those obtained at 50 C (Third Semi-Annual Report, 1 July 1965 - 31 December 1965) it appears that temperature has a direct effect on the properties of ultrasonic waves. Irradiations at 50 C and 60 C yielded 4 and 3 statistically significant values with respect to reductions in viable counts. At 30 C, on the other hand, a total of 7 statistically significant values was obtained. These results support the concept that the antimicrobial activity of air-borne ultrasonic waves is enhanced by lower temperatures.

4. Effect of Propylene Oxide and Ultrasonic Irradiation on Spores of *B. subtilis* var. *niger*.

In the experiments being reported, propylene oxide was used in place of ethylene oxide. Initial efforts were directed toward finding an optimal combination of ultrasonic irradiation time and propylene oxide concentration for most effective and reliable sterilization. Accordingly, spores of *B. subtilis* var. *niger* were exposed to various levels of propylene oxide alone and propylene oxide plus ultrasonic irradiations for different periods of time. Numerous trials were made and the data given represent average values.

Based on our experience with ethylene oxide, a concentration of 250 mg/liter of propylene oxide was selected for our initial experiments. Table 3 summarizes the data obtained with propylene oxide (250 mg/liter) alone and with ultrasound at 60 C:

Table 3. Average counts of spores of *B. subtilis* var. *niger* exposed to propylene oxide (250 mg/liter) alone, and in combination with ultrasound (34.8 kc/sec) at 60 C for varying periods of time.

Exp. No.	Period of Exposure, minutes	Without Sound	With Sound
1	20	500,400	47,435
2	40	77,807	59

Irradiation distance = 1 inch

To determine the role of temperature in the process of sterilization with propylene oxide, experiments similar to those shown in Table 3 were planned using a temperature of 40 C instead of 60 C. The results are shown in Table 4.

Table 4. Average counts of spores of *B. subtilis* var. *niger* exposed to propylene oxide (250 mg/liter) alone, and in combination with ultrasound (34.8 kc/sec) at 40 C for varying periods of time.

Exp. No.	Period of Exposure, minutes	Without Sound	With Sound
1	40	729,000	79,000
2	60	312,000	31,000
3	80	268,000	44,000

Irradiation distance = 1 inch

Comparing the data in Tables 3 and 4 for the 40 minute exposure period, it appears that the lethal action of propylene oxide is favored by higher temperatures. This tentative conclusion also applies to the combination of propylene oxide plus sound.

To determine if an increase in propylene oxide concentration would significantly affect the data obtained thus far, the level of this gas was increased to 500 mg/liter and the temperature was maintained at 40 C. Table 5 illustrates the results obtained.

Table 5. Average counts of spores of *B. subtilis* var. *niger* exposed to propylene oxide (500 mg/liter) alone, and in combination with ultrasound (34.8 kc/sec) at 40 C for varying periods of time.

Exp. No.	Period of Exposure, minutes	Without Sound	With Sound
1	40	833,000	44,000
2	60	401,500	3,400
3	80	378,400	1,228

Irradiation distance - 1 inch

Comparing the data in Tables 4 and 5, it is possible to gain some insight on the effect of increasing the propylene oxide concentration while maintaining all other variables constant. It may be seen that the effect of propylene oxide alone at either 250 or 500 mg/liter, respectively, did not result in appreciably lower counts. The addition of sound, however, reduced the viable count more effectively when coupled with a higher concentration of propylene oxide. An observation worthy of note here, was the fact that much less fluctuation resulted between individual determinations.

To further reduce the degree of fluctuation noted between individual counts, a final series of experiments were run in which the exposure times were increased to a full two hours. Propylene oxide levels of 250 and 500mg per liter, respectively, were employed and the temperature selected was 40 C.

Where sound was employed, the irradiation distances were varied between one and 9.5 inches. Table 6 shows the results obtained using propylene oxide in a concentration of 250 mg/liter:

Table 6. Average counts of spores of *B. subtilis* var. *niger* exposed to propylene oxide (250 mg/liter) alone, and in combination with ultrasound (34.8 kc/sec) at 40 C for 120 minutes at various irradiation distances.

Without Sound	With Sound, Distance from Transducer (inches)			
	9.5	5.0	3.5	1.0
82,296	2,825	3,500	2,081	462

Table 7 illustrates the results obtained when the propylene oxide level was increased to 500 mg/liter.

Table 7. Average counts of spores of *B. subtilis* var. *niger* exposed to propylene oxide (500 mg/liter) alone, and in combination with ultrasound (34.8 kc/sec) at 40 C for 120 minutes at various irradiation distances.

Without Sound	With Sound, Distance from Transducer (inches)			
	9.5	5.0	3.5	1.0
32,181	2,200	1,884	1,114	0

The data in Tables 6 and 7 show that doubling the level of propylene oxide from 250 to 500 mg/liter reduced the viable count by more than 50%. The addition of sound to either level of propylene oxide brought about significant decreases in the count. Further, ultrasound coupled with the higher propylene oxide concentration proved to be the most effective means of sterilization accomplished thus far. The importance of the irradiation distance is well illustrated in both Tables 6 and 7. As was the case in previous work reported,

the closer the sample to the transducer, the greater the kill. Total sterilization was achieved at a distance of 1 inch using propylene oxide (500 mg/liter) coupled with ultrasound (34.8 kc/sec) for a period of 120 minutes. Individual fluctuations in the latter experiment were greatly reduced and it now appears that reliable conditions for achieving sterilization using propylene oxide and ultrasound in combination can be described.

#### 5. Discussion.

The work reported here indicates the importance of considering air-borne ultrasonic irradiations as a potential tool for specific sterilization problems. The use of ultrasound alone significantly reduces viable counts of B. subtilis var. niger. Of interest is the fact that the antibacterial effects of sound alone are most evident at lower temperatures. In addition, the closer the specimen to the sound source, the greater will be the kill.

The use of propylene oxide in our present experiments has permitted us to establish certain base lines for achieving total sterilization. Consideration must be given to the concentration of propylene oxide employed, the period of exposure, and the irradiation distance when ultrasound is added. It has been shown that the combination of propylene oxide and ultrasound results in more effective kill than when either agent is used alone. Of importance in our present effort is the fact that reproducible results can now be achieved more readily.

#### 6. Future Effort.

Experiments employing propylene oxide in combination with ultrasound will continue. It is planned to further vary the propylene oxide levels employed as well as the exposure times. Similarly, the effect of temperature and the distance at which irradiations are made will also be evaluated.

As the above experiments are progressing, it is also planned to initiate a series of studies using the thermophilic organism Bacillus stearothermophilus. It is expected that the use of a thermophile will be of value in assessing the role of temperature in the propylene oxide-ultrasound experiments. The use of B. stearothermophilus will make it possible to compare the behavior of two different species in our sterilizing system.

#### 7. Publication.

A paper entitled "Sterilizing effects of high intensity air-borne sonic and ultrasonic waves" has been accepted for publication. Five preprints are enclosed with this report as well as a copy of the letter of acceptance for publication.